


# The Endocytic Membrane Trafficking Pathway Plays a Major Role in the Risk of Parkinson's Disease

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## ABSTRACT

**Background:** PD is a complex polygenic disorder. In recent years, several genes from the endocytic membrane-trafficking pathway have been suggested to contribute to disease etiology. However, a systematic analysis of pathway-specific genetic risk factors is yet to be performed.

**Objectives:** To comprehensively study the role of the endocytic membrane-trafficking pathway in the risk of PD.

**Methods:** Linkage disequilibrium score regression was used to estimate PD heritability explained by 252 genes involved in the endocytic membrane-trafficking pathway including genome-wide association studies data from 18,869 cases and 22,452 controls. We used pathway-specific single-nucleotide polymorphisms to construct a polygenic risk score reflecting the cumulative risk of common variants. To prioritize genes for follow-up functional

studies, summary-data based Mendelian randomization analyses were applied to explore possible functional genomic associations with expression or methylation quantitative trait loci.

**Results:** The heritability estimate attributed to endocytic membrane-trafficking pathway was 3.58% (standard error = 1.17). Excluding previously nominated PD endocytic membrane-trafficking pathway genes, the missing heritability was 2.21% (standard error = 0.42). Random heritability simulations were estimated to be 1.44% (standard deviation = 0.54), indicating that the unbiased total heritability explained by the endocytic membrane-trafficking pathway was 2.14%. Polygenic risk score based on endocytic membrane-trafficking pathway showed a 1.25 times increase of PD risk per standard deviation of genetic risk. Finally, Mendelian randomization identified 11 endocytic membrane-trafficking

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**Funding agencies:** This research was supported, in part, by the Intramural Research Program of the National Institutes of Health (National Institute on Aging, National Institute of Neurological Disorders and Stroke; project numbers: project numbers 1ZIA-NS003154, Z01-AG000949-02, and Z01-ES101986). In addition, this work was supported by the Department of Defense (award W81XWH-09-2-0128) and The Michael J. Fox Foundation for Parkinson's Research.

**Relevant conflicts of interest/financial disclosures:** Mike A. Nalls' participation is supported by a consulting contract between Data Tecnica International and the National Institute on Aging, NIH, Bethesda, MD, USA, as a possible conflict of interest. Dr. Nalls also consults for SK Therapeutics Inc, Lysosomal Therapeutics Inc, the Michael J. Fox Foundation, and Vivid Genomics, among others.

Full financial disclosures and author roles may be found in the online version of this article.

**Received:** 3 October 2018; **Revised:** 5 December 2018; **Accepted:** 23 December 2018

Published online 00 Month 2018 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.27614

pathway genes showing functional consequence associated to PD risk.

**Conclusions:** We provide compelling genetic evidence that the endocytic membrane-trafficking pathway plays a relevant role in disease etiology. Further research on this pathway is warranted given that critical effort should be

made to identify potential avenues within this biological process suitable for therapeutic interventions. © 2019 International Parkinson and Movement Disorder Society

**Key Words:** endocytosis; genetic risk; heritability; Parkinson's disease; polygenic risk score

Parkinson's disease (PD) is a progressive neurodegenerative disorder clinically characterized by the manifestation of motor disturbances such as bradykinesia, rigidity, tremor, and a wide range of nonmotor symptoms. Disease is caused by the death of the dopaminergic neurons in the SN with the consequent deficit of striatal dopamine.<sup>1</sup> The etiology of PD is complex, including genetic, epigenetic, and environmental factors. Familial PD accounts for less than 10% of the total cases, mostly attributed to monogenic forms with autosomal-dominant (including *SNCA*, *LRRK2*, and *VPS35*) or autosomal-recessive pattern of inheritance, such as *PARKIN*, *PINK1*, and *DJ-1*, among others.<sup>2,3</sup> However, the vast majority of the disease is considered to be sporadic, likely caused by the cumulative effect of multiple common or rare variants of small or moderate effect sizes in addition to other unknown environmental and stochastic factors.

Over the last two decades, extensive work in genetics has uncovered up to 92 common risk loci associated with idiopathic PD.<sup>4</sup> As expected from complex diseases, the PD-related signals identified to date only account for a small proportion of the total phenotypic variation, defined as heritability.<sup>5</sup> It is estimated that only around 10% to 15% of the overall heritable risk of PD is explained by current genome-wide association studies (GWAS).<sup>4</sup> The most plausible explanation for the “missing heritability” might be small effect sizes of the yet to be discovered risk loci which do not reach GWAS significance, as well as structural genomic and rare variation poorly covered by current technology.<sup>6</sup> Undoubtedly, the estimated heritability in PD suggests that a significant amount of risk has not been uncovered yet.

Aligned with the genetic discoveries, a big effort has been made to identify how those genes interact within biological pathways to elucidate the cellular and molecular processes that could explain the neurodegenerative mechanism.<sup>7</sup> Recent findings point out that dysfunction in the endosomal membrane-trafficking pathway (EMTP) could be a driving force in the pathogenesis of PD.<sup>8</sup> The EMTP is a complex multistep pathway that involves different process and organelles. First, cells uptake macromolecules and other cargo from the extracellular space to a group of organelles called early endosomes. From this point, internalized molecules can recycle back to the plasma membrane directly or through recycling endosomes. Alternatively, endocytosed

cargo can be retained in early endosomes that will eventually mature into late endosomes and fuse with lysosomes to ensure cargo degradation. In parallel, proteins can also traffic between endosomes and the Golgi apparatus (for a review, see a previous work<sup>9</sup>). A substantial amount of genetic variation in PD and parkinsonism previously nominated genes has been associated with vesicle trafficking by endosomal gene alterations,<sup>10</sup> including monogenic genes (*VPS35*,<sup>11</sup> *DNAJC6*<sup>13,18</sup>) and GWAS-nominated genes (*RAB7L1*,<sup>12,13</sup> *SH3GL2*,<sup>14</sup> *GAK*,<sup>15,16</sup> and *CHMP2B*<sup>15,17</sup>). In addition, other well-known PD genes, such as *LRRK2*<sup>12,13</sup> and *PLA2G6*,<sup>18</sup> seem to be linked to the aforementioned pathway through their biological interaction with EMTP genes. *LRRK2* binds to *RAB7L1*<sup>12,13</sup> and *PLA2G6* interacts with *VPS35* and *VPS26*, although a clear role of these genes in the EMTP is yet to be elucidated. The genes involved in the EMTP play different roles within the pathway. For instance, *DNAJC6*, *SYNJ1*, *GAK*, and *SH3GL2* are linked to clathrin-coated vesicles whereas *VPS35*, *DNAJC13*, or *RAB7L1* participate in recycling components from the endosomes to the Golgi. Hence, it seems plausible that other genes in that pathway could be involved in PD risk and therefore account for the nonreported heritability.

Based on these premises, we aimed to benefit from a cellular perspective to systematically explore the genetic contribution of 252 endosomal membrane-trafficking-related genes on the susceptibility for PD. We set out to study to what extent common variation in these genes might play a role on the genetic architecture of PD by performing genome-wide heritability estimations and polygenic risk versus disease status and age at onset in the International Parkinson's Disease Genomics Consortium (IPDGC) GWAS data set. We also applied Mendelian randomization to attempt to investigate possible functional genomic associations between nominated variants of interest within this pathway versus expression and methylation quantitative trait loci. Finally, we used biological pathway analysis and expression data from murine SN to point out the biological contribution of the genetic findings.

## Patients and Methods

### Subjects

The IPDGC GWAS data set consisted of 41,321 individuals (18,869 cases and 22,452 controls) of European

ancestry. Clinical and demographic characteristics of the cohorts under study are shown in Supporting Information Table S1. Additional details of these cohorts can be found in Nalls and colleagues.<sup>4</sup>

No individuals with known pathogenic mutations were excluded from following analyses. When the case age at onset or control age at last exam was not provided, individuals were likewise included.

### Genes Selection and Pathway Analysis

The EMTP is a general and extense pathway that includes several subpathways. EMTP-related genes under study were selected by using The Molecular Signatures Database (MSigDB) of annotated gene set for “endocytosis”<sup>19,20</sup> based on the Kyoto Encyclopedia of Genes and Genomes information (KEGG-endocytosis). However, the gene list provided by KEGG was not completely accurate and failed to represent some relevant genes within the EMTP. In order to validate whether each gene flagged as endocytosis was involved in the EMTP, a detailed literature search was performed. In addition, because the current research in the EMTP is very dynamic and there are constantly new discovered proteins involved in some aspects of the pathway, we included relevant genes that have been proved to participate in the EMPT, but were not nominated by KEGG. In addition, a thorough review of the literature was performed using the terms “endocytosis,” “endosome to Golgi retrograde transport,” “endosomal maturation,” and “endosomal recycling.” We have provided a table (Supporting Information Table S2) that includes each gene involved in our analysis with the literature source that refers to. A total of 262 genes were flagged as “endocytic membrane-trafficking” (Supporting Information Table S2), of which 252 were autosomal and 10 spanned in chromosome X. The latter were excluded for the following analyses because of lack of data. The PANTHER web-based Classification System was used to obtain the Top 10 significant enriched Reactome Pathways with an adjusted false discovery rate (FDR)  $P$  value  $<0.05$ . Additionally, we obtained expression data from single-cell RNA sequencing performed in 243 putative dopaminergic neurons at post-natal days 28 to 56 from Slc6a3-Cre/tdTomato mice (GSE76381).<sup>21</sup> We applied an arbitrary selected threshold of expression. Only genes with total expression values across the 243 cells higher than 100 were nominated as expressed. Human genes were converted to their ortholog genes using Mouse Genome Informatics (MGI; <http://www.informatics.jax.org>; Supporting Information Table S3). For Venn diagram visualization, the online-based website <http://bioinformatics.psb.ugent.be/webtools/Venn/> and Inkscape software (version 0.92.2; <https://inkscape.org>) were used.

### Quality Control

#### Genome-Wide Association Data

Quality-control (QC) procedures in the IPDGC GWAS data set have been previously described elsewhere.<sup>4</sup> In brief, samples with call rates  $<95\%$  and whose genetically determined sex from X chromosome heterogeneity did not match that from clinical data were excluded. Individuals exhibiting heterozygosity greater than 6 standard deviations (SDs) from the population mean and samples of non-European ancestry were not included. Individuals related at more than the level of first cousin estimated from linkage pruned genetic data on common variants were excluded (1 proband from any family cluster was extracted randomly). Regarding variant QC, variants exhibiting Hardy-Weinberg equilibrium  $P$  value  $<1E-5$  and missingness rates  $>5\%$  as well as palindromic single-nucleotide polymorphisms (SNPs) were excluded from further analyses. Data were imputed using the Haplotype Reference Consortium (<http://www.haplotype-reference-consortium.org>), under default settings with phasing using the EAGLE option (<https://www.nature.com/articles/ng.3643> <https://www.ncbi.nlm.nih.gov/pubmed/27571263>). VCFs were filtered for imputation quality  $>0.30$  for inclusion in analyses.

### Heritability Analysis

In order to estimate the phenotypic variance of PD explained by genes in the extended EMTP pathway, we estimated heritability using linkage disequilibrium (LD) score regression (LDSC).<sup>22,23</sup> We used LDSC to estimate heritability in this study because it requires only summary-level data and is more computationally efficient for larger data sets. We extracted EMTP regions of interest from a meta-analysis of GWAS data and then ran LDSC under default settings. Reference LD scores were computed with the European ancestry subset of the 1000 Genomes data for SNPs within 500 kilobase pairs (kb) of the SNP to be scored.

First, we estimated heritability explained by variants in the whole set of EMTP genes and missing heritability by excluding previously PD-associated, EMTP-related genes. Subsequently, to evaluate significant enrichment of heritability within our pathway of interest compared to any similar set of genes, we resampled 50 random sets of 252 genes in the same sample series (after excluding EMTP genes and regions  $\pm 250$  kb from PD GWAS loci). During resampling, we implemented the exact sample LDSC analyses and extracted the heritability per sampling iteration. Next, we calculated the mean and SDs of the heritability across sampling iterations. Finally, the mean heritability of our resampled series was compared to the test set to determine whether our previous estimates were significantly

divergent from random sets of genes of similar size using a *t* test.

In an attempt to explore the genes that contribute the most to the estimated heritability, we performed the Sequence Kernel Association Test (SKAT) in imputed data adjusting by age, sex, and data subset membership without applying any frequency filtering. Significance was evaluated using 10,000 permutations at  $\alpha = 0.001$ .

### Polygenic Risk Profiles Versus Disease Status and Age at Onset

For the risk-profiling analysis, we mirrored the workflow used in the most recent PD meta-analysis to date,<sup>4</sup> with the exception that before running this analysis pipeline, we extracted only genotypes from the pathway of interest. In brief, PRS profiling is calculated traditionally based on weighted allele dose manner as per previous work.<sup>25-32</sup> We used the R package PRSice2.<sup>33</sup> Permutation testing and *P*-value aware LD pruning was used to identify best *P* thresholds in external GWAS data to construct the PRS, allowing us to utilize variants below the common GWAS significance threshold of  $5E-08$ . External summary statistics utilized in this phase of analysis included data from leave-one-out meta-analyses that exclude the study in which the PRS was being tested, avoiding overfitting/circularity to some degree. LD clumping was implemented under default settings (window size = 250 kb;  $r^2 > 0.1$ ), and for each data set, 10,000 permutations were used to generate empirical *P* estimates for each GWAS-derived *P* threshold ranging from  $5E-08$  to 0.5, at a minimum increment of  $5E-08$ . Each permutation test in each data set provided a Nagelkerke's pseudo  $r^2$  after adjustment for an estimated prevalence of 0.005 (aged population estimate as per Gasser and colleagues)<sup>33</sup> and study-specific eigenvector 1 to 5, age, and sex as covariates. GWAS-derived *P* threshold with the highest pseudo  $r^2$  was selected for further analysis. Summary statistics were meta-analyzed using random effects (restricted/residual maximum likelihood) per study-specific data set by using the R package PRSice2.<sup>34</sup>

For the age at onset risk profiling, we performed similar analyses with the exception that we used age at onset as a continuous variable.

### Mendelian Randomization Analysis to Explore Pathway-Specific Quantitative Trait Loci

We utilized two-sample Mendelian randomization to investigate possible functional genomic associations between nominated variants of interest in the EMTP pathway versus expression and methylation patterns. Quantitative trait loci (QTL) association summary statistics across well-curated methylation and expression data sets from the SMR (<http://cnsgenomics.com/software/smr>)<sup>35</sup> were compared to PD outcome

summary statistics<sup>4</sup> after extracting the pathway-specific variants considered as the instrumental variables. These include estimates for methylation and cis-expression from the Genotype-Tissue Expression (GTEx) Consortium (v6; whole blood and 10 brain regions),<sup>36</sup> the CommonMind Consortium (CMC; dorsolateral prefrontal cortex),<sup>37</sup> the Religious Orders Study and Memory and Aging Project (ROSMAP),<sup>38</sup> and the Brain eQTL Almanac project (Braineac; 10 brain regions),<sup>39</sup> as reported elsewhere. Furthermore, we studied expression patterns in blood from the largest eQTL meta-analysis so far.<sup>40</sup>

Wald ratios were generated for each of the instrumental variable SNPs tagging a cis-QTL (probes within each gene and meeting a QTL *P* value of at least  $5E-8$  in the original QTL study) and for a methylation or expression probe with a nearby gene. Linkage pruning and clumping were carried out using default SMR protocols. With each data type, *P* values per instrument substrate (gene-level expression summaries for eQTLs and cis-gene probe level for mQTLs) were adjusted by FDR.

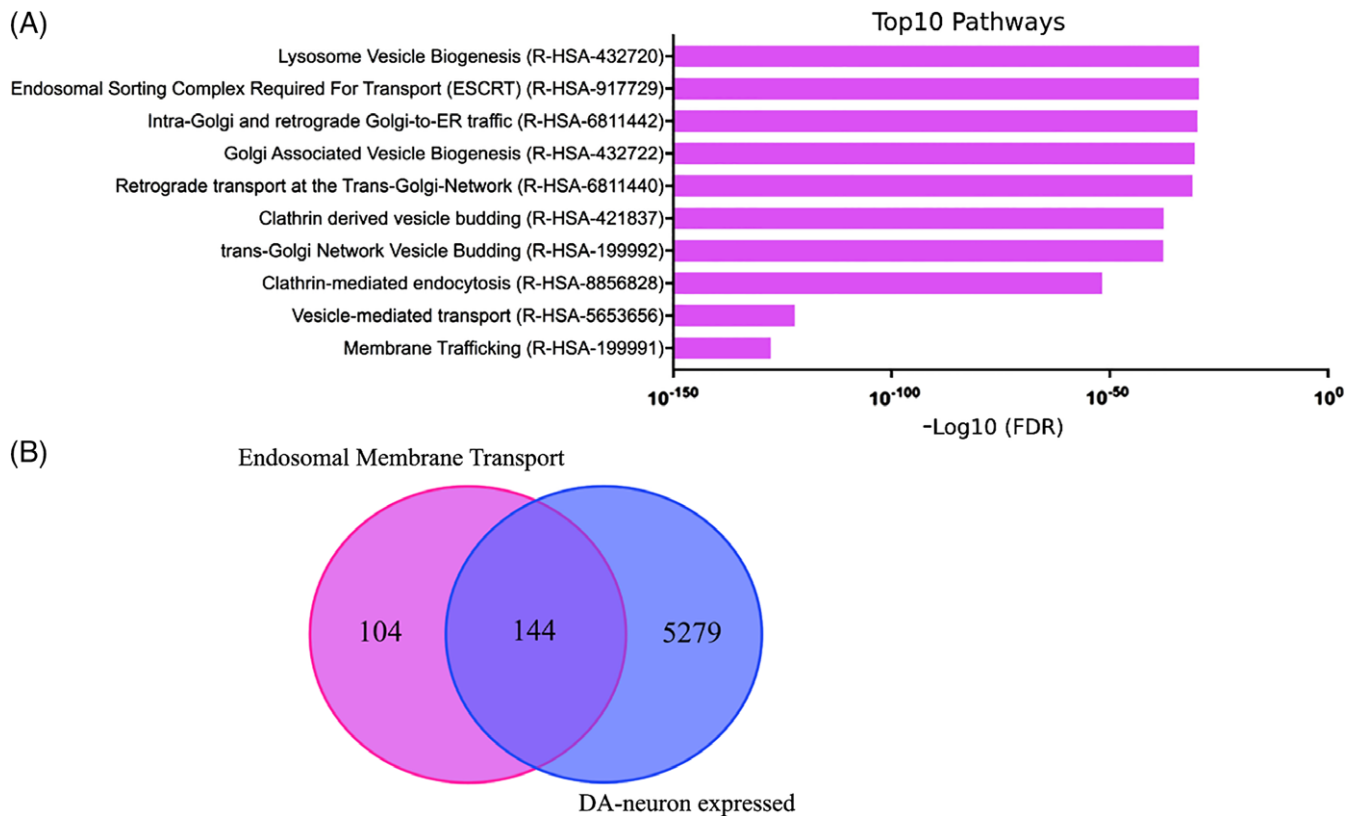
## Results

### Pathway Analysis and Expression of EMTP-Related Genes

EMTP variants from imputed data were extracted from 252 genes selected as described in the Patients and Methods section. Distribution of variants within the EMTP in the IPDGC cohort are highlighted in Supporting Information Table S4. Gene Ontology analysis was performed on these genes showing an enrichment for trafficking-related biological processes such as lysosomal biogenesis, clathrin-mediated endocytosis, retrograde transport at the *trans*-Golgi network or vesicle-mediated transport among others (Fig. 1A). Remarkably, 151 genes among the analyzed genes were found expressed in dopaminergic neurons in adult mouse brains (Fig. 1B).

### Heritability Analysis

We measured heritability explained by the 252 genes under study. EMTP genetic contribution to PD heritability was estimated to be 3.58% (standard error [SE] = 1.17). After excluding previously nominated PD and parkinsonism genes related to the EMTP, including *DNAJC13*, *VPS35*, *DNAJC6*, *SYNJ1*, *SYT4*, *SYT11*, *GAK*, *RAB7L1*, *CHMP2B*, and *SH3GL2*, missing heritability explained by EMTP-related genes was estimated to be 2.21% (SE = 0.42). Reported heritability estimates might be underestimated given that there are possibly rare variants within these genes poorly covered by current genotyping technologies or lost after imputation. These estimates were significantly different from 50 randomly generated sets of 252 genes (*t* test,  $P < 2.2E-16$ ) giving a heritability estimate of 1.44%



**FIG. 1.** Biological implication of EMTP genes in dopaminergic (DA) neurons. (A) Top 10 significant Enriched Reactome Pathways with an adjusted FDR  $P$  value  $<0.05$ . Pathway enrichment analysis was performed using Panther. Only the top 10 enriched pathways were selected for visualization. (B) Overlap between genes expressed in DA neurons and EMTP gene set. The EMTP human genes were transformed in their mouse homolog genes. From the 252 total human genes, a total of 244 were found in mouse (Supporting Information Table S3). DA neuron gene expression was obtained from GSE76381<sup>21</sup> after an arbitrary selected threshold of expression. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

(SD = 0.54) and showing that the heritability enrichment in the EMTP pathway is not likely by chance (Supporting Information Fig. S1). After subtracting the randomly generated heritability estimates (1.44%) that might have biased our initial EMTP estimates, the EMTP genetic contribution to PD heritability was estimated to be 2.14%. When considering 22.9% as the latest estimate of total heritability for PD using the same methodological approach,<sup>4</sup> the EMTP explains roughly 9.34% of the overall heritability of PD.

Additionally, we explored what genes contributed to a greater extent to the PD heritability estimates within the EMTP. Nineteen genes surpassed multiple test correction after the SKAT analysis with FDR-adjusted  $P$  values  $<0.05$  (Supporting Information Table S5). Only *GAK* has been previously associated with PD.

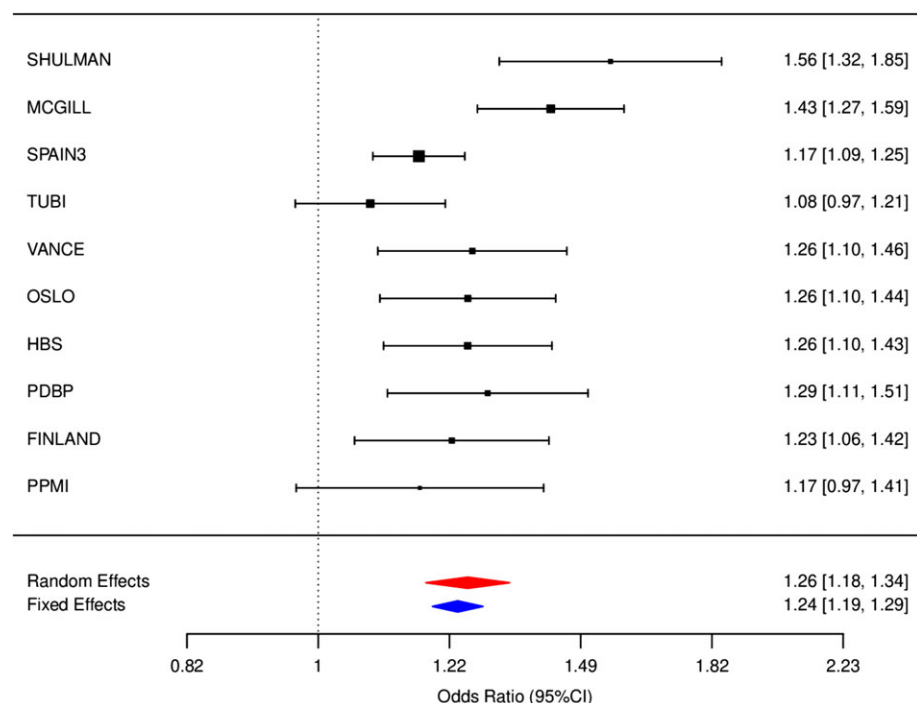
### Polygenic Risk Profiles Versus Disease Status and Age at Onset

EMTP risk profiling versus disease status was found to be significantly associated with PD (random-effects  $P$  value =  $2.55\text{E-}12$ ; beta = 0.227; SE = 0.032) and an odds ratio of 1.20 per SD increase in the PRS from the population mean (see Fig. 2 for forest plot showing

effect estimates within gene sets). Heterogeneity was estimated at an  $I^2$  of 59.32% across all arrays. In all data sets, the directionality of effect was similar. The data obtained from the different data sets strongly enhance the general conclusion for our study. EMTP risk profiling versus age at onset was not found to be significantly associated (random-effects  $P$  value = 0.182).

### Mendelian Randomization Analysis to Explore Pathway-Specific QTL

We also tested a number of QTL resources across varied tissues of interest to make functional inferences relating to biological features that might be involved in the genetic etiology of the disease. After adjustment for FDR correction, 11 EMTP-related genes showed functional consequence by two-sample Mendelian randomization in expression and methylation datasets (see Table 1). Increased blood expression of *VAMP4*, *ARL8B*, and *GAK* was found to be inversely associated to PD risk, whereas a positive risk association was found for *RABGEF1*, *VAMP8*, *CLTCL1*, and *ITSN1*. Increased brain expression of *SH3GL2* and *GAK* was causally linked to PD risk whereas *ARL8B* expression



**FIG. 2.** Polygenic Risk Score across EMTP. Forest plots of PRS estimates across cohorts. For each effect estimate, the size of the square is proportional to the size of the study, with 95% confidence intervals as horizontal error bars. The random-effects estimates are represented by the red diamond, with fixed effects in blue, and the centerline of each diamond representing the summary PRS estimate. The number of samples per cohort are reported as follows: SHULMAN: 789 cases and 195 controls; MCGILL: 583 cases and 906 controls; SPAIN3: 2,120 cases and 1,333 controls; TUBI: 741 cases and 944 controls; VANCE: 621 cases and 303 controls; OSLO: 476 cases and 462 controls; HBS: 541 cases and 743 controls; PDBP: 543 cases and 284 controls; FINLAND: 386 cases and 493 controls; and PPMI: 363 cases and 165 controls. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

exhibited a negative association. mQTL Mendelian randomization analyses revealed eight CpG sites linked to PD risk after multiple test correction. Increased methylation of *GAK* and *HSPA1B* was found to be positively associated with disease whereas *PLEKHM1* and *VPS39* showed a negative directionality versus PD risk.

## Discussion

The present study provides compelling evidence for EMTP variation in PD risk. Using a set of pathway-specific genes/variants, we demonstrate that the phenotypic variance attributed to this complex process contributes to the heritability of PD outside of what is explained by GWAS.

Genetic variance in PD has been estimated to be ~22.9%,<sup>4</sup> and to date only a small proportion of the total heritability has been explained.<sup>4</sup> Our data show that approximately 2.14% of the common variant heritability in PD can be explained by genetic variants in the EMTP, explaining roughly 9.34% of the overall estimates in PD. Here, we have only included the transcribed genes assuming the limitation that the heritability residing in regulatory regions adjacent to the up- or downstream of these genes is not included in this estimate. The reported estimates suggest that there are additional PD genes within the EMTP yet to be

discovered. Our results are in concordance with the largest and most recent meta-analysis in PD, which tested up to 10,651 biological pathways. Among these, only 27 were enriched for PD associations after multiple testing correction,<sup>4</sup> and, notably, endosomal trafficking was included with significant *P* value.

PRS has previously demonstrated how cumulative small effect variants can contribute to PD risk and age at onset.<sup>30</sup> PRS has emerged as a probabilistic tool to infer how independent loci with small effects can be collapsed into a single numerical value useful in predicting the risk of a disease. It has been successfully used to dissect the genetic complexity of brain disorder and quantify risk in many common complex conditions<sup>42,43</sup> for early detection, prevention and therapeutics.<sup>44,45</sup> Here, we assessed the overall cumulative contribution of EMTP-specific loci/genes in PD risk through PRS calculations. Our data show that the PRS based on 252 EMTP gene regions is associated with a 1.2 times increased of PD risk per SD, confirming a cumulative pathogenic effect of EMTP variants in PD.

When assessing whether the EMTP had any effect on the age at onset (AAO), we did not find any significant association. PD genetic studies using AAO as a predictor report greater genetic burden with earlier AAO.<sup>29,46</sup> Linking specific phenotypic aspects of PD to pathways constitutes a critical effort, but remains challenging given that larger longitudinal cohorts of well clinically



**TABLE 1.** Significant functional associations for EMTP-related genes by two-sample Mendelian randomization

Gene	Probe	Beta	SE	P Adjusted	Data Source	Analyte	Top QTL SNP	Chr	Bp
<i>VAMP4</i>	ILMN_1761363	−0.121	0.022	5.21E-06	Expression <sup>40</sup>	Blood	rs10913529	1	171679465
<i>VAMP4</i>	ILMN_1804676	−0.156	0.029	5.21E-06	Expression <sup>40</sup>	Blood	rs11580522	1	171720978
<i>ARL8B</i>	ILMN_1752837	−0.174	0.045	4.80E-03	Expression <sup>40</sup>	Blood	rs4499578	3	5167618
<i>GAK</i>	ILMN_1813775	−0.090	0.025	1.12E-02	Expression <sup>40</sup>	Blood	rs6964	4	843184
<i>RABGEF1</i>	ILMN_2230577	0.266	0.080	1.94E-02	Expression <sup>40</sup>	Blood	rs4718424	7	66376095
<i>VAMP8</i>	ILMN_2190084	0.041	0.012	2.20E-02	Expression <sup>40</sup>	Blood	rs1058588	2	85808871
<i>ITSN1</i>	ILMN_1718769	0.077	0.025	3.66E-02	Expression <sup>40</sup>	Blood	rs2251854	21	35056247
<i>CLTCL1</i>	ILMN_1694584	0.066	0.022	3.88E-02	Expression <sup>40</sup>	Blood	rs2854643	22	19189706
<i>SH3GL2</i>	ENSG00000107295	0.252	0.045	2.45E-06	Expression <sup>41</sup>	Brain	rs10756899	9	17684784
<i>GAK</i>	ENSG00000178950	0.508	0.103	3.47E-05	Expression <sup>41</sup>	Brain	rs11248057	4	906131
<i>ARL8B</i>	ENSG00000134108	−0.033	0.010	1.90E-02	Expression <sup>41</sup>	Brain	rs6787725	3	5201054
<i>GAK</i>	cg14517359	0.158	0.030	1.51E-05	Methylation <sup>41</sup>	Brain	rs3775127	4	886061
<i>HSPA1B</i>	cg00970279	0.048	0.010	4.99E-05	Methylation <sup>41</sup>	Brain	rs506770	6	31785228
<i>HSPA1B</i>	cg16237409	0.051	0.011	4.99E-05	Methylation <sup>41</sup>	Brain	rs506770	6	31785228
<i>PLEKHM1</i>	cg06925179	−0.094	0.026	4.92E-03	Methylation <sup>41</sup>	Brain	rs4523963	17	43570226
<i>GAK</i>	cg23992470	0.093	0.027	1.20E-02	Methylation <sup>41</sup>	Brain	rs114066161	4	825635
<i>PLEKHM1</i>	cg04703951	−0.144	0.043	1.20E-02	Methylation <sup>41</sup>	Brain	rs4523963	17	43570226
<i>VPS39</i>	cg04726019	−0.092	0.028	1.28E-02	Methylation <sup>41</sup>	Brain	rs57393446	15	42494536
<i>GAK</i>	cg26978381	0.119	0.037	1.38E-02	Methylation <sup>41</sup>	Brain	rs2306253	4	884475

P adjusted = adjusted false discovery rate P value after performing Mendelian randomization between QTL resources (exposures) versus Nalls and colleagues<sup>4</sup> (outcome).  
Abbreviations: Chr, chromosome; Bp, base pair.

characterized patients are necessary. We believe that the PRS approach we report on here should be further explored in other clinical outcomes related to PD.

Growing evidence has suggested that abnormalities in endosomes or dysregulation in their trafficking plays an important role in several neurological diseases such as Alzheimer's disease or Lewy body dementia.<sup>47,48</sup> This pathway is emerging as key to understanding the mechanisms underlying both protein degradation and neurodegeneration. We performed SKAT analysis in order to nominate the genes that contribute the most to the risk of PD within the EMTP. Nineteen genes were significant after FDR correction, including *PLEKHM1*, *IQSEC3*, *GAK*, *VPS39*, *AMPH*, *SMURF1*, *CHMP1A*, *AP2B1*, *VPS28*, *VAMP8*, *AP3B2*, *RABGEF1*, *PLEKHG7*, *TBC1D15*, *AP4E1*, *AP2M1*, *STX8*, *ARF-GAP2*, and *AP3M1*. Interestingly, nine of them are involved in cargo degradation and eight of them play a role in cargo uptake, which strongly suggests that these two subpathways are specially relevant for PD risk. Only *GAK* has already been associated with PD risk through GWAS,<sup>4,30,31</sup> and for the remaining 18 genes not identified in previous studies, only five, including *IQSEC3*, *VPS39*, *CHMP1A*, *AP3B2*, and *AP4E1*, have been associated with neurological disorders. For instance, *IQSEC3* protein expression is downregulated in the brain of patients that suffer from autism and schizophrenia,<sup>49</sup> and loss-of-function mutations in *CHMP1A* cause pontocerebellar hypoplasia and microcephaly.<sup>50</sup> Autosomal-recessive mutations in *AP3B2* cause early-onset epileptic encephalopathy,<sup>51</sup> and a mutation in *AP4E1* has been found in a familiar case of spastic paraplegia.<sup>52</sup> Furthermore, QTL analysis using

Mendelian randomization indicates that five genes among the ones nominated by SKAT showed functional relevance associated with PD (*PLEKHM1*, *VPS39*, *GAK*, *VAMP8*, and *RABGEF1*).

The present study shows that EMTP genes contribute to PD risk. However, we are aware of some limitations. First, our work is restricted by the sensitivity of array-based genotyping methods used to calculate heritability. Likewise, heritability estimates might be biased, appearing lower than what should be expected. The remaining variance in PD etiology not ascribed to the EMTP-identified heritable factors suggests a contribution of joint effects of rare and structural variants poorly covered by current genotyping technology. Therefore, further comprehensive studies are needed to identify novel variants within the EMTP that are relevant for disease. Additionally, there may be some selection bias in the PRS, although the meta-analysis suggests consistent effect estimates across studies.

The endosomal trafficking membrane system is a complex and dynamic process where internalized proteins, receptors, and ligands interact and move between organelles in order to fulfill the cell's needs. Here, we point to PD, at least to some extent, as an endosomal membrane-trafficking disease. This study demonstrates the contribution of this unknown pathway to the oligogenic nature of PD and highlights the importance of pathway-based comprehensive approaches as a way to prioritize functional studies. Following the most recent meta-analysis,<sup>4</sup> pathway analysis established that the nominated candidate genes were linked to autophagy, mitochondrial biology, immune response, and lysosomal function. We envisage that our methodology

could be applied to remaining relevant pathways in PD as a way to better understand genetic risk factors. Unraveling the genetics underlying PD will provide with therapeutic options for drug discovery and ultimately could lead to the development of effective interventions. ■

**Acknowledgments:** We thank all of the subjects who donated their time and biological samples to be a part of this study.

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## Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.